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Effects of 4-aminopyridine on nystagmus and vestibulo-ocular reflex in ataxia-telangiectasia

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Abstract:

Ataxia-telangiectasia (A-T) is a progressive neurodegenerative disorder with prominent eye movement deficits localizing to the cerebellum. We sought to determine if 4-aminopyridine (4-AP) that putatively enhances the precision of Purkinje neurons, could improve the disorders of eye movements and vestibular function in A-T.

The influence of 4-AP on disorders of eye movements and vestibular function was studied in four A-T patients. The effects on the cerebellar control of vestibulo-ocular reflex (VOR) was quantitatively assessed by the decay time constant of per- and post-rotational nystagmus during constant velocity en bloc rotations. The length of the VOR time constant determines the fidelity of the vestibular velocity storage, a neural mechanism that increases the bandwidth of VOR under cerebellar control. The VOR time constant was not increased in A-T patients. Latter is explained by the extent of cerebellar lesion as previously described in A-T and other cerebellar disorders. Nevertheless, 4-AP shortened the VOR time constant during horizontal rotations. Severe disinhibition of velocity storage in subjects with putatively profound cerebellar degeneration manifest periodic alternating nystagmus (PAN). Among two A-T subjects who manifested PAN, 4-AP reduced the peak slow-phase velocity of the more severely affected individual and abrogated the PAN in the other. Two A-T subjects manifested horizontal and vertical spontaneous nystagmus (SN) in primary gaze, 4-AP reduced its slow-phase velocity.

We conclude that in subjects with A-T 4-AP has a prominent effect on the ocular motor and vestibular deficits that are ascribed to the loss of cerebellar Purkinje neurons.

Introduction:

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by neural degeneration, immunodeficiency, radiosensitivity, and enhanced risk of chiefly lymphoreticular malignancy. Neuropathologic features found in individuals with A-T are focused in the cerebellar cortex, with diffuse loss of Purkinje and granule cells. Early investigators thus classified this disease as a cerebellar ataxia syndrome and gave A-T its iconic name [1, 4, 6-8, 13, 23]. Many of the neurologic deficits apparent in individuals with A-T, such as gaze-evoked nystagmus, periodic alternating nystagmus (PAN), ocular motor apraxia, impaired smooth pursuit, saccadic dysmetria, ataxia, and kinetic tremor, point to degeneration of the cerebellar cortex [4, 7, 20, 24, 25, 32, 33].

We sought to determine if 4-aminopyridine (4-AP) can improve the deficits of eye movement and vestibular function in subjects with A-T. 4-AP is a blocker of inward rectifier potassium currents [11]. One proposed consequence of 4-AP treatment is facilitation of synchrony and thus greater precision of Purkinje neuron discharge [2]. An effect on discharge precision may facilitate the synaptic release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) at Purkinje neuron projection sites [2, 14, 16, 29]. This mechanism has been offered as a possible explanation for how 4-AP decreases the downbeat nystagmus in individuals having disorders of the cerebellum [14, 16, 29].

We hypothesized that 4-AP could improve the deficits that localize to the cerebellar cortex in A-T subjects.

Methods:

Effects of 4-AP was assessed in four subjects with A-T. All four subjects had a mutation in the A-T associated ATM gene (McConville et al, 1996), and a diagnosis of A-T by accepted clinical criteria [9, 21]. Table 1 depicts the summary of their clinical presentation. Subjects were evaluated at the vertigo center and vestibulo-oculomotor laboratory of the University Hospital Zurich, Switzerland. 10 mg 4-AP, which was prescribed to the patients to decrease their cerebellar deficits, was first administered during the clinical visit. Its efficacy was assessed by quantitative measurements of the eye and limb movements and the VOR. The assessment protocol was approved by the local ethics committee. All subjects signed a written informed consent form.

Experimental setup

Subjects were seated on a three-axis motor-driven turntable (Acutronic, Jona, Switzerland). The head was stabilized by a custom-molded thermoplastic mask (Sinmed BV, Reeuwijk, The Netherlands). Three-dimensional eye movements were recorded with dual search coils (Skalar Instruments, Delft, The Netherlands). The search coil annuli were calibrated and then placed on the sclera after local anesthesia with oxybuprocaine 0.4% [5]. The coil frame around the head (side length: 0.5 m) generated three orthogonal digitally synchronized magnetic wave field signals at 80, 96 and 120 kHz. A digital signal processor computed a fast Fourier transform in real time on the digitized search coil signal to determine the voltage induced on the coil by each magnetic field (system by Primelec, Regensdorf, Switzerland). Coil orientation could be determined with an error of less than 7% over a range of ±30°, and with a noise level of less than 0.05° (root mean squared deviation). Eye position signals were digitized at

1,000 Hz per channel with 12-bit resolution. Calibrated eye position signals were processed with an interactive program (MatLab®; The Math Works, Natick, MA, USA). Eye positions were represented as 3D rotation vectors in a head-fixed coordinate system.

Vestibulo-ocular reflex measurement

The VOR was elicited during constant velocity *en bloc* rotations in a completely dark room. Rotation with an initial acceleration at 90º/s^2 continued to a final speed of 100º/s. Eye movements were then recorded for 90 seconds of rotation time. The turntable was then decelerated at 90º/s^2 until it stopped, and eye movements were measured for 90 seconds. These rotational trials were done with the subject in three different positions. In an upright posture, the rotation primarily stimulated the horizontal semicircular canal (yaw rotation); rotations with the subject supine (roll rotation), or recumbent left ear down (pitch rotation), primarily stimulated the vertical canals. Because the rotations were around the earth vertical axis gravitational force applied to the inner ear remained constant. All rotations were in both directions (i.e. in head-centered frame of reference right or left for yaw rotations, up or down for pitch rotations, and clockwise or counterclockwise for roll rotations). Each subject thus experienced at least six sets of constant velocity rotations, one in each direction of each of the three axes. Each trial was separated by a rest period of at least two minutes.

Assessment of gaze-holding

Eye movements were recorded when the subjects attempted to hold gaze steady at straight ahead on space fixed laser target for at least two to three minutes. During

assessment of gaze-holding function, the vestibular chair was secured such that the subject remained in the upright position.

Data analysis

Rotation vectors were smoothed, and angular eye velocity of nystagmus was computed as described previously [28]. The time constant of the decaying slow phase eye velocity was computed in steps. First, the slow-phase velocity of nystagmus versus time was plotted. Velocity was then fitted according to an exponential function. Least-squares fitting algorithm (Matlab®, Mathworks™, Optimization Toolbox) was used to compute the exponential time constant that best fit the data.

Median slow phase velocity of each nystagmus cycle was utilized to sample a cohort of slow phase velocities during given fixation trial. The epochs of slow phase velocities for the given patient during horizontal, vertical, and torsional nystagmus was used to assess statistical significance of the drug effect; hence for the measurement of drug effect, each patient served as self-control. In subjects with PAN, the slow phase eye velocity of nystagmus modulated in sinusoidal fashion. Sinusoidal function was fitted to quantify the periodicity and the amplitude of PAN. Peak to peak time determined the duration of period, while the peaks and trough values of the sinusoidal fit yielded maximum slow-phase velocities of PAN in each direction.

Results:

Effects of 4-AP on vestibulo-ocular reflex (VOR):

VOR time constant

We measured the effects of 4-AP on the decay time constant of the velocity storage to assess its influence on the VOR. Parallel, direct and indirect, pathways guarantee efficient function of VOR [22]. The direct pathway, comprised of three-neuron-arc, is responsible for rapid reflexive changes in eye velocity in response to head movement [3]. The indirect pathway features the neural integrator, the velocity storage, that stabilizes the eye velocity during whole field rotation and extends the band-width over which the VOR is compensatory for head movement [22]. Gamma-aminobuteric acid (GABA) mediated inhibition through the Purkinje neurons of cerebellar nodulus and ventral uvula determines the fidelity of the velocity storage, its function is measured by the decay rate of the velocity storage output, the slow phase eye velocity of rotational nystagmus during en bloc constant velocity rotations [31]. It is therefore anticipated that cerebellar disease might affect the velocity storage, while drugs influencing the cerebellar function might affect the decay time constant of the velocity storage. The drug that increases the inhibitory influence of Purkinje neurons, such as 4-AP, shortens the velocity storage time constant [26]. The velocity storage time constant is typically measured by assessing the rate of decay of slow-phase eye velocity of per-rotational nystagmus during *en bloc* constant velocity rotation. The rate of decay of slow-phase eye velocity of post-rotational nystagmus immediately after the rotation is also used to measure the velocity storage time constant.

Figure 1 illustrates the effect of 4-AP on the slow-phase velocity of the rotational nystagmus in one subject. The slow-phase velocity of the horizontal nystagmus during yaw rotations had an abrupt rise that was immediately followed by an exponential decay (Figure 1A). Before 4-AP the time constant of decay was 11 seconds (grey symbols, Figure 1A), while 30 minutes after taking 10 mg 4-AP the time constant was 8 seconds (open circles in Figure 1A).

Figure 1B and 1C depict examples of torsional and vertical nystagmus during roll and pitch rotations around the earth-vertical axis. The slow-phase velocity of the torsional nystagmus during roll rotations decayed at a time constant of 6.8 seconds before 4-AP, the decay time constant was 6.5 seconds after 4-AP (Figure 1B). The decay time constant of vertical nystagmus during pitch rotations before and after 4-AP remained unchanged at 6.2 seconds (Figure 1C).

The decay time constants of per- and post-rotational nystagmus were analyzed in three of the four subjects. One subject was excluded from this analysis because periodic alternating nystagmus (PAN) interfered with computation of decay time constant. Figure 2 depicts the summary, decay time constant before 4-AP is plotted along x-axis, while y-axis represents the decay time constant after 4-AP. Each data point depicts one rotation trial, each symbol type depicts individual subject; the dashed line is an equality line. Figure 2A illustrates summary of yaw rotations. Circular and diamond shaped data points fall below the equality line, while triangular data points fall on the equality line. These results suggest that 4-AP consistently reduced decay time constant of rotational nystagmus during yaw rotations in two subjects; in one subject 4-AP had no effect. Figure 2B depicts a summary of effects of 4-AP on decay time

constant of torsional nystagmus during roll rotations. All data points fall along the dashed equality line, suggesting lack of 4-AP effect on the decay time constant of torsional VOR. Likewise 4-AP had no effect on decay time constant of vertical VOR evoked during pitch rotations, all data points in Figure 2C fell along the dashed equality line.

The same technique to measure eye movements and the same parameters of vestibular stimuli in healthy subjects revealed mean horizontal VOR time constant at 13.6 seconds with variance of 16.2 seconds [26]. The mean decay time constant for torsional and vertical VOR during roll and pitch rotations in healthy subjects were 6.3 seconds and 7.8 seconds (variance: 1 second and 4.8 seconds), respectively [26]. As evident from the summary in Figure 2A, the decay time constant of horizontal, vertical and torsional VOR during yaw, pitch, and roll rotations were not increased. The mean value of horizontal, vertical, and torsional VOR time constants were 9.2 ± 2.5 seconds, 6.7 ± 1.2 seconds, and 5.8 ± 1.5 seconds, respectively. 4-AP reduced the population mean of horizontal VOR during yaw rotations to 6.4 ± 1.6 seconds (31.5% reduction), however, vertical and torsional VOR time constants had minimally changed to 6.5 ± 1.3 seconds (3% reduction) and 5.2 ± 1.4 (9% reduction) seconds, respectively.

VOR gain

The VOR gain reflects the ratio of peak eye velocity to peak head velocity. Although ideal value of VOR gain should be one, in most healthy subjects the VOR gain in darkness is typically less than one. Using current experiment design and paradigms, the VOR gain in healthy subjects was 0.55 ± 0.2, 0.43 ± 0.14, and 0.34 ± 0.16 during yaw, pitch and roll rotations respectively [24, 26]. In A-T subjects however, the VOR

gain is higher than healthy subjects [24]. VOR gain in our subjects was measured by computing the ratio of peak slow phase eye velocity immediately after the onset of head rotations and rotational head velocity. Figure 3 illustrates the summary of VOR gain measured from A-T patients. Each data point depicts the VOR gain during one rotation trial, circular symbols depict yaw rotation, square symbols are pitch rotation, while triangles depict roll rotation. VOR gain measured after 4-AP is plotted on y-axis, while x-axis depicts the values of VOR gain during control condition. Dashed line is an equality line. All data points fall along the equality line. The slope of the fitted linear function relating the values of gain before and after 4-AP was 0.91. The gain values before and after 4-AP intake significantly correlated (correlation coefficient: 0.89; $p < 0.0001$). Mean VOR gain in our A-T subjects was 0.99 ± 0.13, 0.6 ± 0.14, and 0.47 ± 0.09 during yaw, pitch and roll rotations respectively. After administration of 4-AP the mean VOR gain was 1.01 ± 0.1, 0.7 ± 0.17, and 0.45 ± 0.08 during yaw, pitch and roll rotations, respectively.

Effects of 4-AP on gaze holding – periodic alternating nystagmus (PAN)

Periodic alternating nystagmus (PAN) is a condition where the nystagmus changes direction in periodic manner. The sequence of PAN includes nystagmus that beats on one side, transition period, reversal of beating direction, and then repetition of the sequence. PAN was present in two A-T subjects. The nystagmus was quantified by measuring median eye velocity during of each nystagmus slow-phase (e.g. each datapoint in Figure 4). Median slow-phase velocity was then plotted with corresponding time, the periodic modulation of nystagmus resulted in sinusoidal shape (Figure 4). The relationship of slow-phase velocity that modulated with time was then fitted with

sinusoidal function. The peak and trough value of sinusoid depicted peak slow-phase velocity of PAN in each direction, while inverse of fitted sinusoid frequency provided the duration of PAN. Peak slow-phase velocity of PAN, before 4-AP, was 23 ± 4 º/second in subject 1 and 4.3 ± 3.0 º/second in subject 2. The periods were 16 ± 3 and 6 ± 3 seconds in subjects 1 and 2, respectively. In subject 1, 4-AP reduced the peak slow-phase eye velocity of PAN to 13 ± 1.3 º/second (43.5% reduction) but did not alter the duration of the period (Figure 4A). 4-AP resolved the PAN in subject 2 (Figure 4B).

Effects of 4-AP on gaze holding –spontaneous nystagmus

Spontaneous nystagmus was present in all three axes. Horizontal, vertical, and torsional nystagmus was quantified independently. Median velocity of the slow phase of each nystagmus cycle was measured. The cohort of median slow phase velocity computed from multiple oscillatory cycles comprising nystagmus was then used for statistical comparison of the effects of 4-AP on nystagmus. Analysis was done only on data collected when the subjects fixated gaze on straight ahead target. Figure 5 summarizes the slow-phase velocity of horizontal, vertical, and torsional nystagmus during straight-ahead fixation. This analysis was not done for either horizontal or vertical nystagmus in subject 1, or for horizontal nystagmus in subject 2, due to the confounding effects of PAN. In Figure 5, each box and whisker plot depicts the summary of population comprising slow phase velocities from all nystagmus cycles in a given direction from one subject. The horizontal line in the center of a notch depicts a population median, notch depicts 95% confidence interval, while the length of the box depicts interquartile interval. 4-AP reduced the slow-phase eye velocity of horizontal nystagmus in one of two (Figure 5A), and for vertical nystagmus in all three (Figure 5B)

subjects (one-way ANOVA $p < 0.05$). The subjects, in whom 4-AP had significant effects the notches of corresponding box-whisker plots (depicting populations of slow phase velocity before and after 4-AP), did not overlap (Figure 5A,B). There is an overlap in notches of box and whisker plots comparing the populations of slow phase velocities before and after 4-AP (Figure 5C). Latter suggested lack of 4-AP effect on the torsional nystagmus on any subject (one-way ANOVA, $p > 0.05$).

Discussion:

4-AP affects measures of impaired gaze holding and vestibular function in subjects with A-T. These deficits have been attributed to reduced GABAergic inhibition upon the brainstem vestibular and deep cerebellar nuclei as a consequence of progressive degeneration of the cerebellar cortex. 4-AP is thought to be effective in cerebellar degenerations as a result of its ability to facilitate Purkinje cell output.

A prominent feature of cerebellar control of eye movements is reflected in low-frequency modulation of the VOR. A 'velocity-storage' mechanism prolongs the decay of the per- and post-rotational nystagmus by means of GABA-mediated inhibitory control from the cerebellar Purkinje neurons in the posterior cerebellar vermis. Individuals with damage to this region, including those with A-T in whom Purkinje neuron dropout is pan-cerebellar, thus manifest disinhibited velocity storage. When severe, disinhibition of velocity storage can manifest with PAN, which emerges in this state as a consequence of an adaptive vestibular mechanism that normally acts to minimize sustained unidirectional nystagmus, even to the point of causing the oscillating reversals of direction [12, 17, 18, 31]. Although we saw PAN in our subjects with A-T, we did not observe an increase in the VOR time constant. This observation is possible, as prolongation of VOR time constant might depend on the extent of lesion as previously reported in individuals with A-T and other cerebellar lesions [15, 24]. As expected, 4-AP reduced the duration of velocity storage time constant in subjects with A-T; the reduction was prominent for yaw rotations, while time constant during pitch and roll rotations were unaffected. This observation is consistent with the effects of 4-AP on velocity storage time constant in healthy subjects [26]. It is predicted that lack of effects

of 4-AP on time constant of torsion and vertical VOR is because of weak or absent contribution of velocity storage during rotation in vertical canal planes [30].

Typical duration of PAN is approximately four to five minutes [19]. The duration of PAN, however, relies on the adaptation time constant [18]. Hence, shorter adaptation time constant could cause short cycle PAN. Short cycle PAN has been previously reported in subjects with A-T [25].

4-AP did not affect VOR gain measured during rotations along any axis. These results are similar to the recent study where 4-AP was injected locally within the cerebellar flocculus or subcutaneously in *tottering* mice featuring CACNA1A mutation [27]. In healthy humans too, the pharmacological manipulations of the velocity storage are known to spare the VOR gain [10, 26]. The VOR gain reflects the state of direct pathway featuring three-neuron-arc [3]. Therefore our results are consistent with previous studies and suggest that effects of 4-AP to modulate VOR function is selectively via the velocity storage.

By diminishing the inward rectifying potassium conductance of an action potential, 4-AP is thought to increase pre-synaptic quantal content across a broad range of central and peripheral neurons. In disease states where neuronal dropout diminishes input to targets below excitation threshold, this action may partially restore selective function. This action is likely possible only over a narrow range of pathology, and hence those with severe degeneration may not manifest improved function.

Our experience with just four subjects having A-T, over a short duration, in a focused neurologic function where available outcome assessment is sensitive and reliable, is nonetheless sufficiently encouraging to suggest that further testing of 4-AP is

warranted. These studies, with other outcome measures, over a longer interval of treatment to access other mechanisms of action, with similar agents having better pharmacokinetics, or a combination of these and other protocol modifications, may well demonstrate a broader range of favorable effects.

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Figure legends:

Figure 1 Vestibular nystagmus is evoked during (rotational nystagmus) and after (post-rotational nystagmus) constant velocity en bloc rotation in complete darkness. Rotations in the horizontal plane (yaw rotations) pre-dominantly evokes horizontal nystagmus, rotations in coronal plane (roll rotations) evokes torsional nystagmus, while sagittal plane rotations (pitch rotations) cause vertical nystagmus. Slow-phase velocity of the per-rotatory nystagmus is plotted versus time. (A) Gray symbols illustrate slow phase eye velocity of the horizontal rotational nystagmus. Notice a rapid rise followed by a slow exponential decay of the slow phase eye velocity of the nystagmus. The decay time constant of the rotational nystagmus in the given example was 11 seconds. The open circles represent the slow phase eye velocity of the horizontal rotational nystagmus, 30 minutes after the same subject took 10 mg 4-AP by mouth. After administration of 4-AP, the decay time constant of the rotational nystagmus in this subject reduced to 8 seconds. (B) Slow phase eye velocity of the torsional rotational nystagmus is illustrated with grey symbols. Administration of 4-AP did not alter the decay time constant of the torsional rotational nystagmus (Before 4-AP: 6.8 seconds; after 4-AP: 6.5 seconds). (C) The decay time constant of the slow phase eye velocity of the vertical rotational nystagmus also remained unchanged on administration of 4-AP (Before 4-AP: 6.2 seconds; after 4-AP: 6.2 seconds).

Figure 2 Effects of 4-AP on the decay time constant of the per- and post-rotational nystagmus in three A-T subjects are summarized. The decay time constant of per- and post-rotatory nystagmus before 4-AP is plotted versus after 4-AP. (A) The decay time

constant of the per- and post-rotational horizontal nystagmus from two subjects was significantly reduced by administration of 10 mg 4-AP (One-way ANOVA, $p < 0.05$). Notice square and circular symbols are below the equality line. However, 4-AP did not change the horizontal rotational nystagmus in one subject (One-way ANOVA, $p > 0.05$). This subject is illustrated as triangular symbols. Notice all triangles fall along the equality line. (B, C) In all three subjects, there was no significant reduction in the decay time constant of torsional and vertical rotational nystagmus as these subjects took 10 mg 4-AP (One-way ANOVA, $p> 0.05$). Notice all symbols representing the decay time constant of torsional rotational nystagmus (B) and vertical rotational nystagmus (C) all along the equality line.

Figure 3 Effects of 4-AP on VOR gain. The values of VOR gain before 4-AP is plotted on x-axis while y-axis depicts VOR gain after 4-AP. Each datapoint depict one rotational trial, while each symbol type represent rotational plane. Dashed line is the equality line. All data point fall along equality line. Values of VOR gain before and after 4-AP relate with correlation coefficient at 0.89 and slope of linear fit at 0.91.

Figure 4 Effects of 4-AP on the peak velocity of the periodic alternating nystagmus (PAN) in two A-T subjects. The slow-phase horizontal eye velocity is plotted versus time. A sinusoidal pattern of the slow phase eye velocity is consistent with periodic alternating nystagmus. Black symbols represent the slow phase eye velocity before 4-AP, while the grey symbols represent the slow phase eye velocity recorded approximately 30 minutes after the subjects took 10 mg 4-AP by mouth. 4-AP

significantly reduced the peak slow phase horizontal eye velocity in one subject, while it completely abolished PAN in the other.

Figure 5 The slow-phase eye velocity of spontaneous horizontal, vertical, and torsional nystagmus when A-T subjects fixated gaze on the central (straight-ahead) target. Black box and whisker plots summarize the slow phase eye velocity before administration of 4-AP, while grey box and whisker plots represent the slow phase eye velocity recorded 30 minutes after these subjects took 10 mg 4-AP by mouth. Horizontal bar in the center of the box and whisker plot represent median slow phase eye velocity, while notches represent 95% confidence interval around median. If notches from two box and whisker plot do not overlap, the difference between their medians is statistically significant (One-way ANOVA, $p<0.05$). In most subjects, 4-AP significantly reduced the slow phase eye velocity of horizontal and vertical nystagmus, but torsional nystagmus was unaffected.

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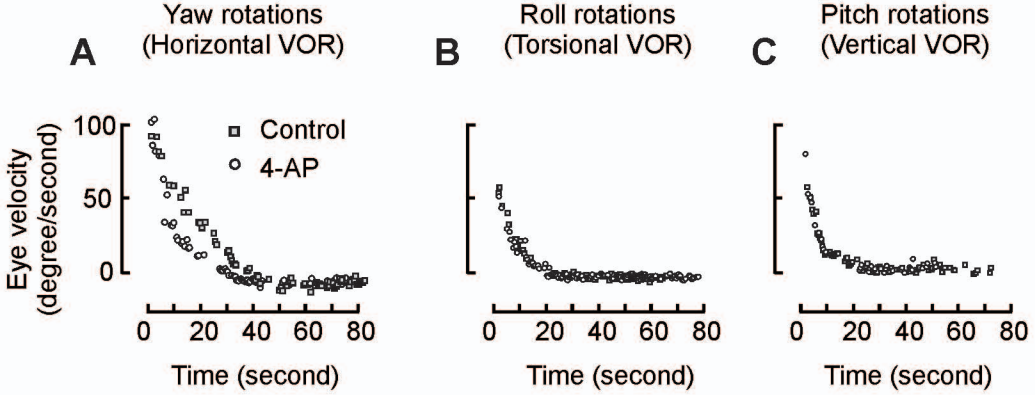
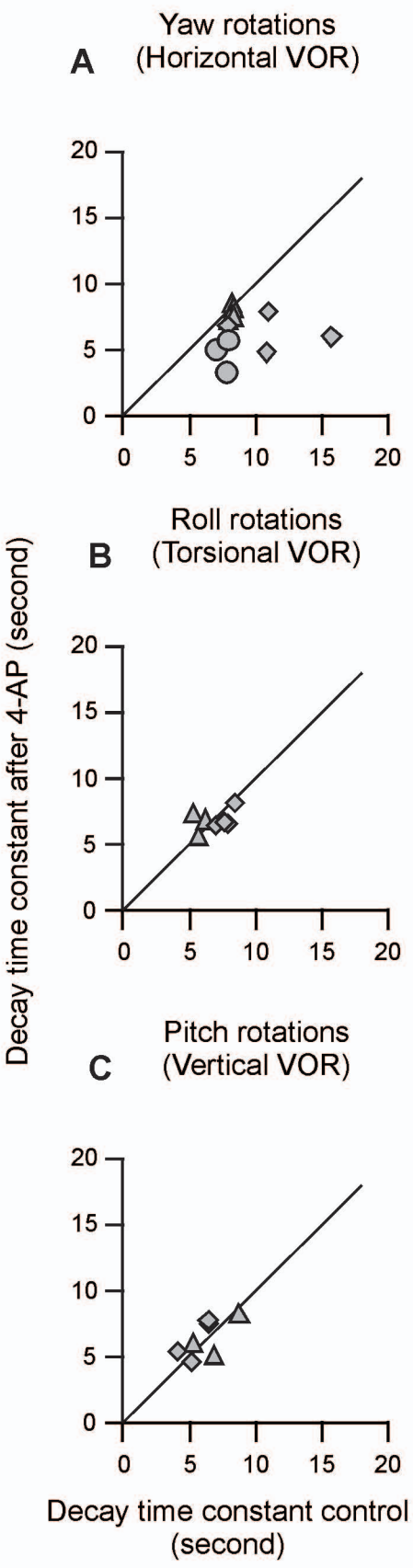
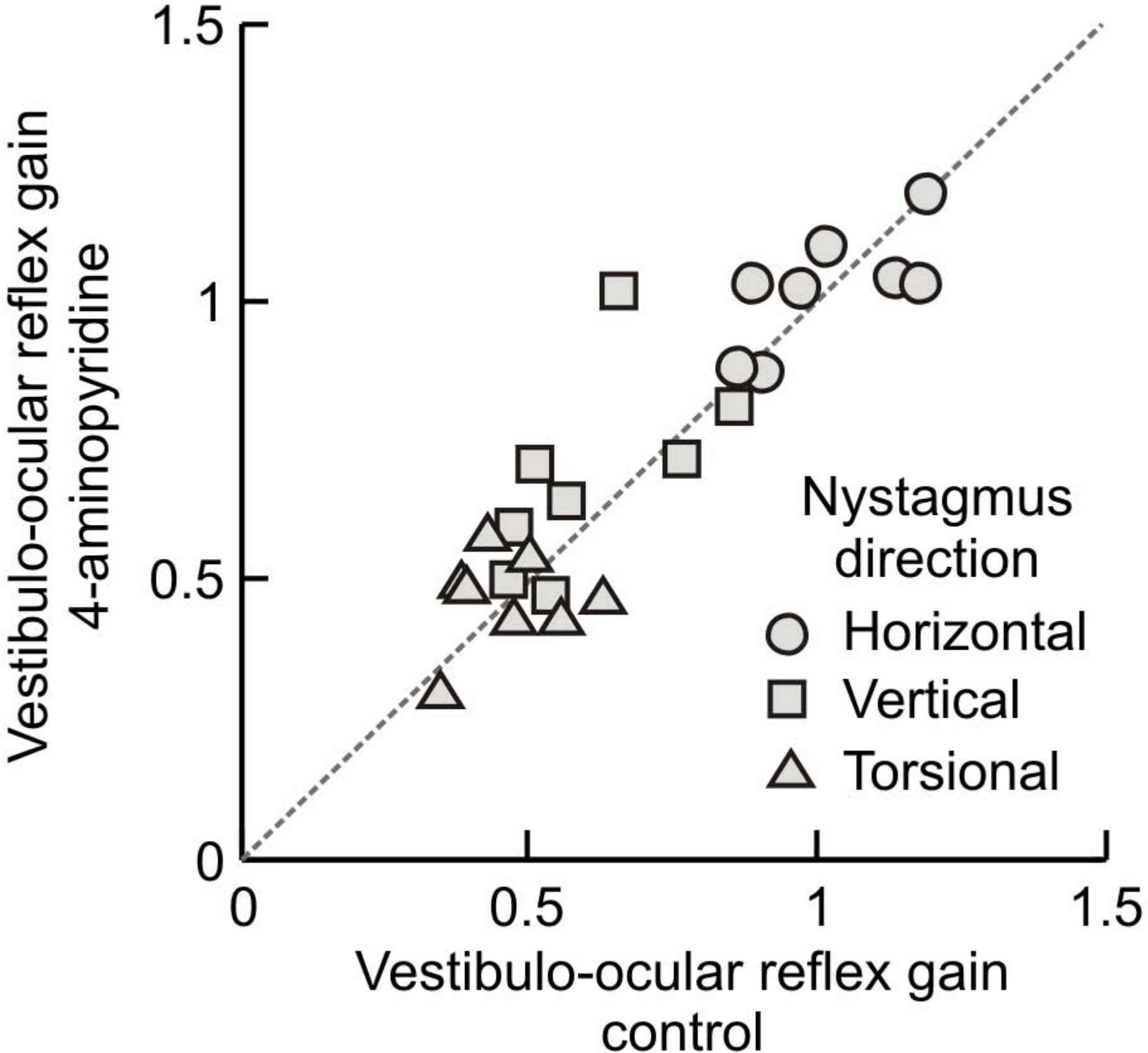
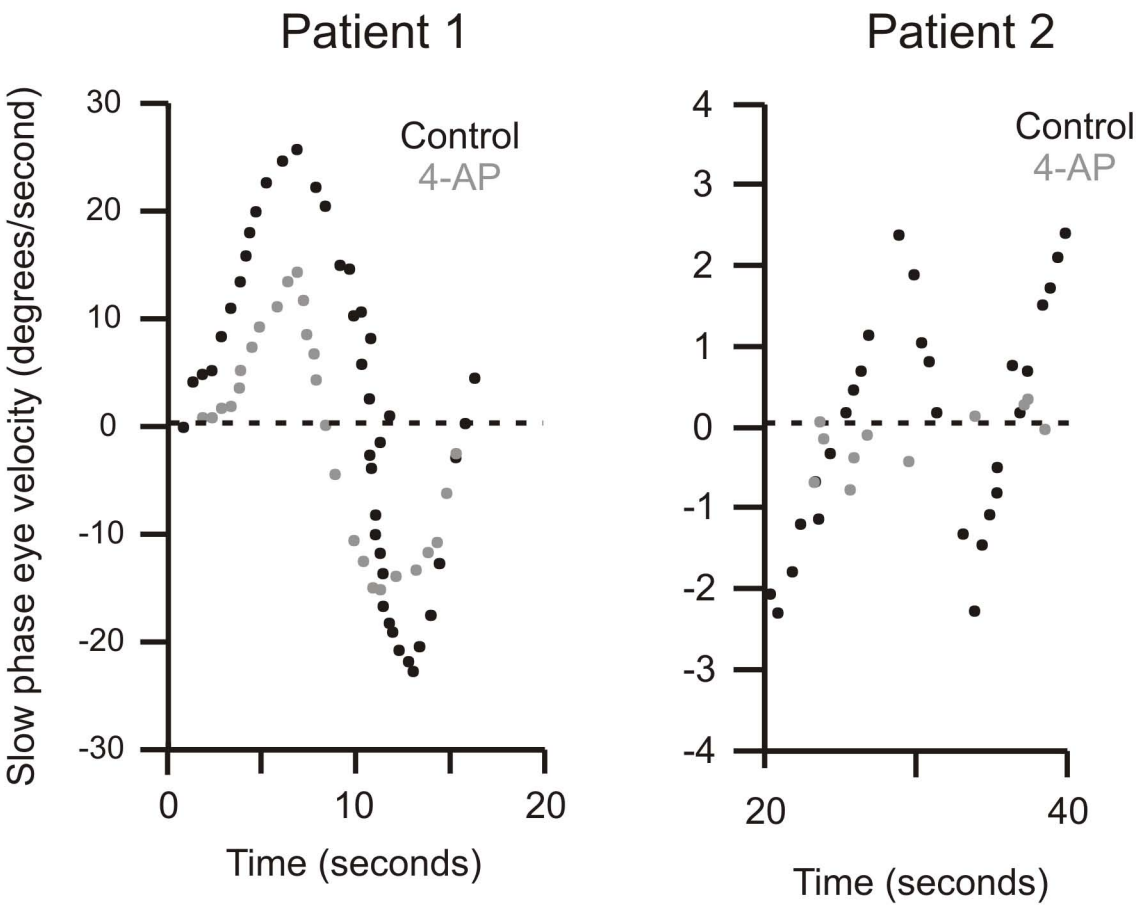
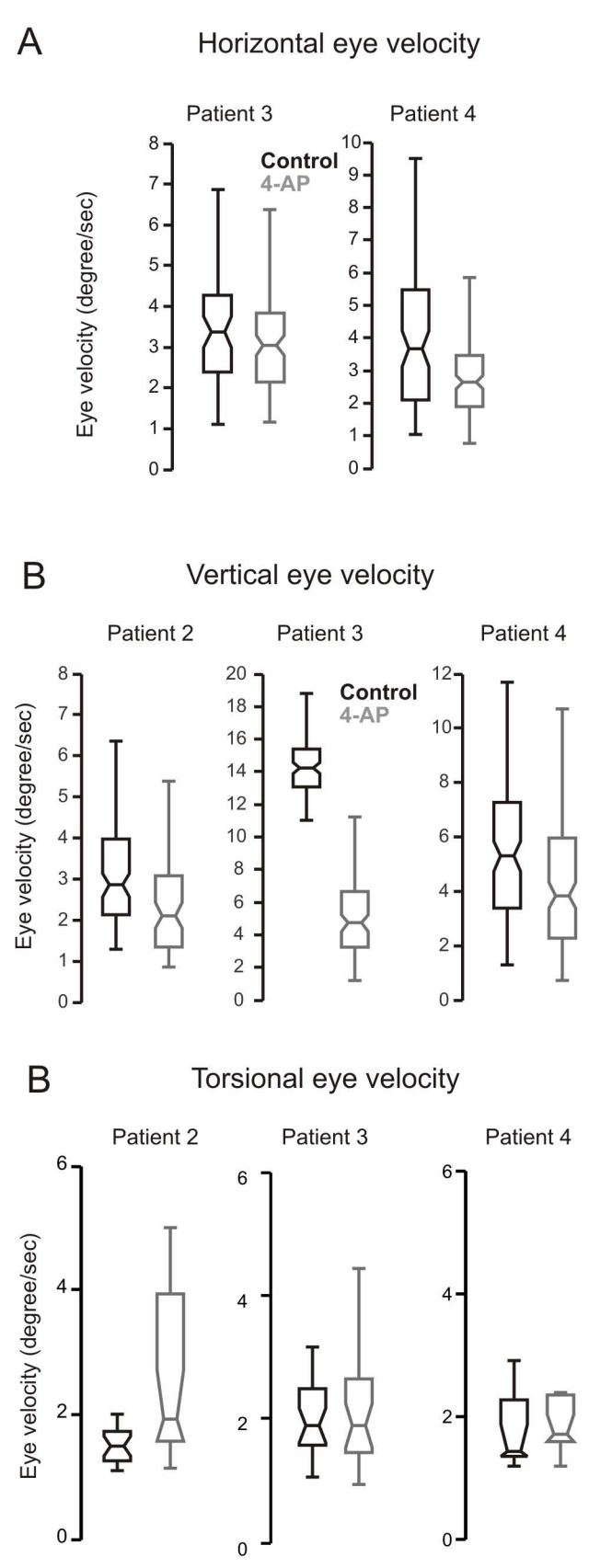

A
Yaw rotations
(Horizontal VOR)
B
Roll rotations
(Torsional VOR)
C
Pitch rotations
(Vertical VOR)
Eye velocity
(degree/second)
100
50
0
Control
4-AP
0
20
40
60
80
Time (second)


A
Yaw rotations
(Horizontal VOR)
B
Roll rotations
(Torsional VOR)
C
Pitch rotations
(Vertical VOR)
Decay time constant after 4-AP (second)
Decay time constant control
(second)
0
5
10
15
20


1.5
1
0.5
0
0
0.5
1
1.5
Vestibulo-ocular reflex gain
4-aminopyridine
Vestibulo-ocular reflex gain
control
Nystagmus
direction
Horizontal
Vertical
Torsional


Patient 1
Patient 2
Control
4-AP
Control
4-AP
Slow phase eye velocity (degrees/second)
30
20
10
0
-10
-20
-30
4
3
2
1
0
-1
-2
-3
-4
0
10
20
20
40
Time (seconds)
Time (seconds)


A
Horizontal eye velocity
Patient 3
Patient 4
Control
4-AP
Eye velocity (degree/sec)
B
Vertical eye velocity
Patient 2
Patient 3
Patient 4
Control
4-AP
Eye velocity (degree/sec)
B
Torsional eye velocity
Patient 2
Patient 3
Patient 4
Eye velocity (degree/sec)